amplification step is to generate about a 0.01 picomolar concentration of detectable nucleic acid from the target molecule. It has been found that this is in the range of the lower detection limit of a sandwich assay with enzymatic amplification and electrochemical detection. The desired one picomolar concentration of fragment is based on Avogadro's number (1 mole=6×10^{23} molecules), where 1 pmol equals $6\times10^{23}\times10^{-12}$, or about 10^{12} molecules. If, as is known, one microliter of blood contains about 5×10^{3} molecules of DNA, then one milliliter, which is a reasonably accessible sample volume, contains approximately 5×10^{6} molecules, or roughly about 10^{7} molecules. To go from the amount of DNA in 1 ml of blood to 0.01 pmol of DNA requires an amplification of about 10^{3} fold. Such an amplification is certainly achievable using several well known amplification techniques. Performing a similar calculation, for a different sample types and sample volumes, to determine the degree of amplification will be apparent to those skilled in the art.

[0095] The polymerase chain reaction (PCR) is well known for its ability to specifically amplify regions of target DNA based on the primer sequences chosen for the PCR reaction. In a preferred exemplary embodiment, a novel method of performing a PCR reaction is used that combines DNA polymerase, a target nucleic acid, and amounts of two modified primers, where the first modified primer has a sequence of bases to a region of the target. A polymerase blocking region is attached to this primer that is linked to a single stranded hybridization region. The second modified primer has a sequence of bases to a second region of the target and also a polymerase blocking region and a second single stranded hybridization region. A detectable moiety (e.g., biotin, fluorocein, or the like) is attached to one or both of the two modified primers. To run the PCR reaction, the mixture is cycled to generate multiple copies of an amplicon incorporating the modified primers. Advantageous to such a method, excess unincorporated modified primers, with the detectable moiety, are substantially eliminated from the final amplicon product. In a preferred method, the primers form a self-annealing hairpin structure that prevents them from interfering in the detection step. In a preferred method, the amplicon product is transferred from the amplification chamber **11** to the detection device **59**, as described above. In the detection device **59**, the amplicon product contacts a capture oligonucleotide that is complimentary to one or both of the single stranded hybridization regions to permit hybridization with the amplicon. In the last step, the moiety associated with this hybridization is detected directly, for example by fluorescent detection of fluorocein. Alternatively, the moiety, e.g., biotin or the like, is exposed to and binds with a streptavidin-labeled enzyme, e.g., alkaline phosphatase or the like, and the enzyme activity is determined either optically or electrochemically.

[0096] The reaction sequence is illustrated in FIG. **14**, where **81** is the detection moiety, e.g., biotin, FAM, DNP, cholesterol, fluorocein, or the like, **82** is the first single stranded hybridization region, **83** is the polymerase blocking region, e.g., hexaPEG or the like, **84** is the first PCR primer, **85** is the second PCR primer, **86** is the second single stranded hybridization region, **87** is a second detectable moiety, **88** is the double stranded nucleic acid target sequence, **89** is a solid substrate, e.g. bead or surface, and **90** is a hybridization region complementary to **86**.

[0097] For a preferred exemplary embodiment, the first and second PCR primers **84** and **85** are preferably synthesized using standard phosphoramidite chemistry, and can include any nucleotide or modified base that is amenable to DNA polymerase, except in the polymerase blocking region **83**. An example of a polymerase blocking region sequence can include the spacer phosphoramidite 18-O-dimethoxyltritylhexaethyleneglycol,1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite. Such a phosphoramidite generates a hexaethyleneglycol spacer region. Other suitable spacer molecules with similar properties can also be used for this purpose. Alternatives to phosphoramidite chemistry can be used, including, but not limited to, creating a 3'-3' or 5'-5' phosphodiester backbone, as well as modified nucleotides as described by Newton, et al. (Nucleic Acids Research 21, pages 1155-62, 1993), and also described in U.S. Pat. No. 5,525,494. The PCR primer also preferably includes a terminal phosphorothioate bond, preventing the exonuclease activity of *T. kodakiensis* KOD1 DNA polymerase from not discriminating allelelic differences in primers used in SNP analysis based on the terminal base being different.

[0098] Allowing PCR to proceed using these synthetic oligonucleotide primers in the presence of the appropriate target and DNA polymerase with associated components generates a newly synthesized DNA molecule with incorporated single stranded regions **82** and **86**. It has been found that while the Taq DNA polymerase can be used, a preferred embodiment uses *T. kodakiensis* DNA polymerase that exhibits a significantly higher turnover number. Such a molecule can then be hybridized by means of **86** to a target sequence **90** on a solid support **89**. The binding moiety region can then be used for generating a signal, for example, by using biotin as the binding moiety and using streptavidin conjugated to a detection enzyme, e.g., horseradish peroxidase (HRP) or alkaline phosphatase (ALP) or the like.

[0099] In a preferred exemplary embodiment, the nucleic acid amplification device is operated as follows: a sample of nucleic acid is collected into the absorbent pad on the wand and introduced into the amplification chamber **11** through the sample entry orifice **16**. It is then screwed into position to seal the orifice. The cartridge is then inserted into the instrument **111** where it engages the electrical and mechanical connection features. In the first step, the instrument applies a force to the fluid pouch **25** causing the fluid to pass out of the pouch **25** and into the amplification chamber **11**, where it is retained by the instrument applying a force to the ingress and egress seals **13** and **15**. The fluid in the chamber **11** causes dissolution of the sugar glass coating of reagents on the silicon wall **17** to form a mixture of sample, buffer, polymerase and primers. Once the electrical connector has engaged the temperature sensor **21** and heating circuit **20**, the cycling of the temperature in the chamber **11** is initiated. The cycling is between a first and second temperature for a predetermined time and for a predetermined number of cycles, as illustrated in FIG. **9**. The fan **48** in the instrument adjacent to the silicon wall **17** of the device **10** provides for the cooling part of the cycle. Once the amplicon is formed in sufficient amount for detection, the instrument releases the force applied to the seals **13** and **15** and opens the ingress **12** and egress **14** to the chamber **11**. The mechanical connector of the instrument then applies a pneumatic force to the pump **24** attached to the ingress **12** and moves the amplicon from the chamber **11** through the egress **14** and into a detection cartridge **59**.